THE
PRAXIS OF URINARY ANALYSIS.

A GUIDE TO THE CHEMICAL ANALYSIS OF URINE.

WITH DIRECTIONS FOR PREPARING ARTIFICIAL PATHOLOGICAL URINES FOR PRACTICING THE VARIOUS TESTS

AND

AN APPENDIX ON THE ANALYSIS OF STOMACH CONTENTS.

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AUTHORIZED TRANSLATION FROM THE AUTHOR'S ENLARGED AND REVISED SECOND EDITION

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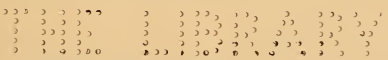

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AUTHOR'S PREFACE TO THE FIRST EDITION.

In the following pages the attempt is made to treat the Analysis of Urine and the Stomach Contents, which are generally given an unjustified exceptional position, in the same manner as a chemical investigation of any other material. We will confine ourselves to the chemical determinations of those constituents of urine and the stomach contents which are of value for diagnosis, following exactly the plan of most text-books on other chemical analyses. Just as the latter do not consider the rare elements, as they are hardly ever encountered in practice, and thereby avoid an excessive complication of methods, we also will omit the determination of the rare constituents and only consider that which experience has shown is sufficient for the practical purposes of the analysis of urine and the stomach contents.

The Author.

Königsberg, 1897.

PREFACE TO THE SECOND EDITION.

In the necessity of giving out a new edition of the book within six months after its first appearance, the author believes he can see a proof of the need of such a book for those circles that have not made and do not make a specialty of urinary analysis.

Munich, 1898.

TRANSLATOR'S PREFACE.

In presenting this little book to the English-reading public the translator feels sure that it will meet with the same favorable reception that it has received in the original language. The author is a practical expert on the subject, and the methods given for analysis embrace all that is necessary for the purposes of the practical physician.

The translator desires to call particular attention to the preparation and use of artificial pathological urines.

Springfield, Ohio, May, 1903.

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ANALYSIS OF STOMACH CONTENTS.

INTRODUCTION.

URINARY analysis, an important contiguous field between medicine and chemistry, is still regarded by a great number of physicians and chemists as a part of their work with which they cannot feel familiar for want of sufficient preparation, since they have only busied themselves thoroughly with one of the above-mentioned sciences. This is certainly deplorable for physicians, and also their patients. The former often thus deprive themselves of their most valuable aid.

There is certainly no lack of text-books on urinary analysis. Some of them are unexcelled for scientific purposes, and it is not the aim of the author to increase their number by writing a similar one. In recent years almost all these books have been written by medical men, or revised by them for many years. Judged by these works, most authors seem to think it a scientific task to make even the simplest urinary analysis. Instead of giving lucid directions for analysis, most of them present a series of innumerable reactions that can be

made with urines containing abnormal constituents. Consequently urinary analysis assumes apparently astonishing dimensions and seems to be only feasible for physicians having a thorough chemical knowledge, but absolutely impracticable for chemists.

The few directions for urinary analysis which are found in some analytical works on general analysis written by chemists are even less intelligible. Some of them are entirely useless, for, according to their directions, it is often quite impossible to decide whether a urine contains, say sugar, or not, to say nothing of other constituents. It is stated in such a work which appeared in 1895 in its sixth edition, and which likewise gives entirely inadequate directions for urinary analysis: "The examination of urine and urinary calculi is a frequently reoccurring work. Its skillful execution brings fame and rich pecuniary reward to the practicing expert." From this can be judged the esteem in which a really reliable urinary analysis is held by many.

The supposition that the chemical analyses of urine and the stomach contents are particularly difficult, which is fostered by all this, is in nowise justified by facts. Let us first inform ourselves as to the object of such analyses on the part of the practitioner. The practicing physician and the chemist desire to know whether a urine contains, for instance, albumen or sugar, or is free from these. The relation is hence

exactly similar to that of the chemist who quite frequently desires to know if a substance contains chlorine. The chemist always* tests for chlorine in the form of silver chloride. He learns this the first day that he takes up the subject of analytical chemistry. The result is that he not only soon masters absolutely this method of detecting chlorine, but he also knows in time the precipitate of silver chloride, its behavior and appearance in such a way that it is impossible for him to mistake it for anything else. Every variation from its common behavior, which points to some other substance, must attract his attention. Of course chlorine can be detected in many other ways than in the form of silver chloride. But no matter to how profitable scientific investigations this study of chlorine reactions can lead, it is and always remains the object of the analyst to be able to say, with infallible certainty, whether a substance investigated by him contains chlorine or not.

In the chemical analysis of urine practical physicians must likewise make up their minds to regard their task in a corresponding manner. Such an analysis, dealing as it does with a liquid, is thereby made very easy in comparison with a general chemical analysis. Physicians must not desire to attempt apparently

* Exceptions are so rare that they need hardly be considered.

scientific investigations with urines in the way of all possible kinds of reactions. Even the best water analysis for practical purposes made by a chemist, for instance the determination of the suitability of a water for drinking purposes, or the analysis of a granite, is in our day no longer regarded as a scientific feat.

If physicians, chemists, and apothecaries will always make their urinary analyses according to one well-recognized method for every single constituent of urine, they also will gain such a confidence in their work, by such continued practice, that they will no longer feel doubtful—and this is a very important point—whether the sought-for constituent is present or not. The paralyzing uncertainty, which is otherwise combined with the distrust of their own analytical results, is then soon lost. They must not try to vary the methods unless they are experts, otherwise the most remarkable things may be found.

For the practice of a physician it is only necessary to prove the presence or absence of the following few substances in urine. This must be emphasized especially for the benefit of chemists who are in the habit of greatly overestimating their number. The urine is tested for albumen, sugar, acetone, acetoacetic acid, bile-pigment, urobilin, blood, indican, and ester sulphuric acids. In certain cases it is not unimportant to test for normal constituents like sulphuric acid, chlorine, etc.

The physician and chemist must also pay attention to the phosphoric acid and ammonia. These will hardly ever have any diagnostical importance, but we will meet with phosphates in testing for abnormal bodies in urine, since they give rise to disturbing secondary reactions. Ammonia will be of interest to us on account of its influence on the real behavior of urines when testing for sugar.

Larger works on urinary analysis must be consulted when urines are encountered which cannot be understood according to the methods herein given. Years may pass before such urines are met with, and some analysts may never come across them.

Urines possessing apparently quite remarkable secondary properties occur after the administration of medicines to patients. In their case also larger works should be consulted. Such urines are seldom found outside of clinics, for most urines of sick persons are generally analyzed before treatment by the physician, who is, of course, supposed to regulate his method of treatment by the result of the analysis.

Practice is hence necessary in urinary analysis the same as in any other reliable analysis. Let the few qualitative methods described later on be practiced until they are completely mastered, which will not take much time, *i.e.*, until convinced to one's own satisfaction that

one feels sure in their manipulation and interpretation. The practice of a physician and apothecary will in time furnish them with the various abnormal urines. In order to afford chemists the *possibility of self-instruction* in places without clinics and hospitals, the author will state under the separate pathological urines how they can be prepared artificially from healthy urines by adding various substances to the latter. Excepting those containing bile-pigments, such artificial urines can be easily prepared by methods which are in part original with the author.

A connected course of instruction in urinary analysis is only possible with the aid of such artificial pathological urines. Each kind cannot be gotten every day even in large clinics. It may be remarked further that physicians often make a microscopical examination of urinary sediments (the solid substances gradually precipitated from urines) besides the chemical analysis. This examination does not offer any particular difficulties as far as the commonly occurring constituents, which are not very numerous, are concerned. The examination of these sediments cannot be discussed here; excellent guides for their microscopical analysis can be found in many text-books on urinary analysis.

The elementary nature of practical urinary analysis (and particularly of the analysis of the stomach contents) and the extreme simplicity of its methods will

mostly surprise chemists, because it does not come up to their expectations. Almost all the tests can be made in a test-tube and permit the presence or absence of the more common pathological constituents to be proven in a few minutes. It must not be supposed that the directions to be given here are too brief. In the course of many years of teaching students and giving courses participated in by old, experienced physicians with a practice of thirty years and more, the author has convinced himself that the following methods of analysis of urine and stomach contents fully suffice for the purposes of the practical physician.

The following may be remarked concerning the methods given for analysis: Methods have been chosen which exclude every ambiguity and are nevertheless as convenient as possible. The so-called boiling method is used, for instance, to detect albumen. The author is well aware that cold tests are also known, which do away with the trouble and time required by boiling urine. Thus nitric acid, acetic acid, and potassium ferrocyanide solution give precipitates with urines containing albumen in the cold; but who can say positively that among all those bodies that can possibly occur in urine, there are not at times some which also give precipitates with nitric acid, acetic acid, and potassium ferrocyanide solution (the latter precipitates many bases). These bodies may come from a food

seldom eaten or from a medicine and may thus give rise to mistaking them for albumen.

The boiling test for albumen excludes all mistakes because dissolved albumen is the only known substance that always coagulates when urine is boiled and then slightly acidified, and hence makes it turbid (see the exceptions, p. 12).

Exempla docent. The author will take the liberty to relate the following personal experience showing to what the use of promiscuous tests, as found in books by the dozen, will lead on the part of those who are unable to properly discriminate. A young physician asked him one day to test a certain urine for albumen. It proved to be free from it. The physician was much astonished, for he had believed that he had found albumen in the urine of his patient for five years. During this whole time the latter showed no unfavorable symptoms, as might be expected from this discovery. He, therefore, had asked the author to make an analysis. When asked how he had tested for albumen, he said that he used for this purpose a solution of picric acid which was especially recommended in English text-books. The acid had always given a precipitate in the urine on standing for some time, which he regarded as showing the presence of albumen. The author could give him this reply: Then you think, perhaps, that you have found albumen in a large part of your rich

practice, while your poor patients must seem much healthier in this regard? He admitted this. The explanation is as follows: Picric acid precipitates albumen; this is undoubtedly true. We shall see how conveniently this fact can be used in quantitative albumen determinations. But the acid also precipitates a normal constituent of urine, creatinine. It combines with this to form a double compound, potassium creatinine picrate, which is practically insoluble in cold urine, and hence gradually precipitated. The physician mistook this slowly formed precipitate for albumen, and as creatinine occurs in richer quantities in urine when meat is eaten than otherwise, the urines of well-to-do people in most cases contain more of this substance than the urine of the poorer classes. The urines of the former, hence, become turbid more easily than those of the latter when picric acid is added, even when albumen is absent.

What little information for diagnostical purposes that can be determined by a chemical analysis of stomach contents will be appended to the urinary analysis. The same can be said of the methods given in the appendix as of those under urinary analysis.

THE PRAXIS OF URINARY ANALYSIS.

I.

URINARY ANALYSIS.

A.

QUALITATIVE TESTS.

1. *Detection of Albumen.*

Fill a test-tube one-third full with the clear urine* (about 8–9 cc. are required) and heat the contents to complete boiling. The urine remains clear or becomes turbid. Now add two or three drops of an approximately 10 per cent. acetic acid and shake. If the urine becomes clear again on this addition, the turbidity is caused by earthy phosphates (see p. 32) and is of no significance. Should it remain cloudy, or should the turbidity increase when the acetic acid is added, albumen is present.

If any doubt exists about a slight cloudiness in a

*Filtered, if necessary.

urine which has been thus treated, the latter is compared with a sample of the unboiled, clear urine in a second test-tube. This comparison will easily show the presence or absence of any cloudiness. This test fails only when the urine is turbid at the start and it is impossible to decide, after boiling and adding acetic acid, whether the turbidity has been increased or not by the precipitation of traces of albumen. In this case also the urine must first be clarified. Ordinary filtration will not suffice, because the fermentation bacteria which cause the turbidity pass through the pores of the filter-paper, the other suspended particles remaining on the filter. Proceed as follows: Place about 2 cc. of crude infusorial earth in a test-tube, fill the latter almost full with urine and shake thoroughly. Now filter through ordinary filter-paper. If the first filtrate is not quite clear pour it back on the filter. An absolutely clear filtrate will soon be obtained. This is divided into two equal parts and the one tested for albumen, as above mentioned. If the boiled, acidified solution is then compared with the other half of the filtrate, it will be easy to detect by comparison even a very slight trace of albumen, as shown by an eventual cloudiness in the boiled urine.

Note. On account of the importance of the albumen test for diagnosis it may be remarked here, for the sake of completeness, that a urine which has been

tested according to this method may appear cloudy although free from albumen, when the patient has used the following internally or externally: turpentine, copaiba, cubeb, santal oil, styrax, or petroleum. The turbidity is then caused by resin acids which have passed into the urine by the use of these remedies and are precipitated by the acetic acid. These resin acids are detected as such by adding considerable alcohol to the boiled urine. They are dissolved by the alcohol; coagulated albumen is not. The author may say that he has never come across such urines although he has made thousands of urinary analyses.

Artificial Albumen Urine.—This is made in the following manner: The white of an egg (about 20 cc.) is diluted with water to 100 cc. and well shaken. When no graduated vessel is at hand the proportion may be estimated by the eye. The liquid is filtered from suspended particles. If 5 drops of such a clear solution are added to 100 cc. of normal urine and the albumen test is made as described, a very perceptible turbidity is produced which differs in no way from that seen in the case of natural albumen urines. 20 drops give a flocculent precipitate, and when 50 drops are taken it is very flocculent, more so than is seen even in natural urines much richer in albumen.

It may be remarked that almost all urines can be kept indefinitely in a stoppered bottle if preserved with

some chloroform, the mixture having been thoroughly shaken. When thus treated, urines will give the reactions unchanged even after the lapse of many years. This is of great advantage to the study of urinary analysis, for urines can thus be kept on hand for practice and comparison.

Artificial Phosphate Urine.—Urines which become turbid on boiling by the precipitation of earthy phosphates (for reasons see p. 32, under Phosphoric Acid) are not met with so frequently as such which do not possess this property. Urines which are voided when much meat is eaten and little is drank are more liable to show this behavior. Any freshly voided urine can be given this property of becoming turbid on boiling, even when albumen is entirely absent, by shaking it with an excess of freshly precipitated calcium carbonate and filtering. The urine will hereby become so rich in lime that it will become turbid on boiling, calcium phosphate being precipitated. This cloudiness is dissolved when acetic acid is added as opposed to the precipitate of coagulated albumen.

The calcium carbonate for this purpose is prepared by adding a solution of sodium carbonate to a solution of calcium chloride in a test-tube. Part of the precipitated calcium carbonate soon settles to the bottom. The supernatant liquid is decanted, more water is added, and shaken. As soon as the precipitate has

again subsided the liquid part is again poured off. Repeat this operation again and then pour the calcium carbonate precipitate into the urine.

In order to learn the manipulation with *infusorial earth* a urine is allowed to stand exposed to the air for several days in an open vessel. Strong decomposition soon occurs. The use only of filter-paper in the usual way will no longer give a clear filtrate with such a liquid. But this can be accomplished by using infusorial earth as directed on page 12.

2. *Detection of Sugar.*

The sugar occurring in the urine of persons afflicted with diabetes is grape-sugar. The best method by far for its detection is the test mentioned by Trommer. In making the test no deviation in any particular must be made from the following directions, otherwise the results will be uncertain. Urines which are to be tested for sugar must be free from albumen, or contain at the most only traces of albumen, since these do not interfere with the test. If they contain any considerable quantity of albumen, a few more cubic centimeters of urine than are necessary for making the albumen test are heated to boiling in a test-tube, two to three drops of acetic acid are added, and the precipitated albumen filtered off. The filtrate which is free from albumen is allowed to cool and then tested by Trommer's method.

Trommer's Test.—Fill a test-tube one-third full with the urine which is free from albumen and then add almost as much of a 10 per cent. sodium hydrate solution (do not be saving with the sodium hydrate solution). The turbidity which is formed by this addition is again due to earthy phosphates (see p. 33), and is of no consequence. Now pour into the mixture, drop by drop, an approximately 5 per cent. copper sulphate solution. The blue precipitate of cupric hydrate which is formed dissolves with a dark-blue color, when grape-sugar is present, as soon as the liquid is shaken. The most important thing about the whole test is that the addition of copper sulphate solution must not be discontinued until the cupric hydrate, which is at first precipitated, is no longer dissolved on shaking and the solution is cloudy from a slight excess of the hydrate, aside from the turbidity caused by the phosphates.

Unless careful attention is paid to the above point the test becomes entirely unreliable when small quantities of sugar are present. This final turbidity which is caused by the cupric hydrate must not be too heavy. With a little practice the proper amount can easily be judged.

Now heat the blue liquid over a Bunsen burner. If yellow clouds of cuprous hydrate * are precipitated

* When Trommer's test is made for practice with an aqueous

before boiling, the presence of sugar is proven; if they appear only on boiling, less sugar is present. If the urine is decolorized, but the yellow cuprous hydrate is not precipitated until after standing for some time, the quantity of sugar in the urine is not very large, according to this manner of making the test. Very slight subsequent precipitations of cuprous hydrate are no proof whatever for sugar, because every normal urine (see p. 43) contains small quantities of reducing substances (viz., uric acid and creatinine reduce under these conditions).

If there is any doubt, after making the test, about the patient having diabetes or not, the following procedure seems best for the practicing physician, according to the author. He permits the patient to partake of a meal which is particularly rich in starch (bread, potatoes, rice) and sugar. The urine voided in the two hours following such a meal is tested by Trommer's method. If this urine also has only slightly reducing properties the patient is not suffering from diabetes. If sugar is found, however, continued investigations will easily decide if it is diabetes or an alimentary glycosuria.

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solution of grape-sugar, red cuprous hydrate is always obtained. Urine containing sugar, on the contrary, gives the yellow modification, except in very rare cases. It is not known what substances in urine give rise to this behavior.

The amount of sugar in the urine of diabetic persons varies very considerably in the course of a day, so long as they do not heed a prescribed diet. Hence in slight cases the morning urine may hardly contain any sugar, and the quantity voided after a meal rich in carbohydrates and sugar still be considerable.

On acount of this fact many physicians make a quantitative as well as a qualitative test for sugar in an average sample taken from the urine voided in the course of twenty-four hours. This plan may also be recommended to chemists.

It may be remarked that every trace of grape-sugar can also be determined with phenylhydrazine. This test excels that of Trommer, but is perhaps too delicate. It requires about a half hour's time and a later examination of the precipitated sediment under the microscope to see if the clusters of the phenylglycosazine are present in the sediment, a test that is considerably more inconvenient for the physician.

Artificial Sugar Urine.—This is made by adding a solution of grape-sugar to normal urine. The latter will also then give the yellow cuprous hydrate. By adding various quantities of the sugar solution it will soon be possible to judge whether a trace, little, or considerable sugar is present in urine, and for practical purposes the phenylhydrazine will not be necessary.

3. *Acetone and Acetoacetic Acid.*

In the later and more severe stages of diabetes acetone, acetoacetic acid, and often oxybutyric acid, besides sugar, are present in considerable quantities in the urine. Only the first two of these substances can be easily detected, and their detection is sufficient for diagnosis.

The oxybutyric acid must be prepared as such in order to prove its presence. This requires at least a week's time and the equipments of a laboratory. The oxybutyric acid can also be recognized by the lævorotation of the urines containing it (see p. 39). For this purpose the urines must first be freed from sugar by fermenting the latter with yeast (see p. 39).

Detection of Acetone.—Pour about two cubic centimeters of water upon a few crystals of sodium nitroprusside contained in a test-tube, and shake until the solution becomes strongly colored. This solution,* which must not be too dilute, is poured into some urine and sodium hydrate is added. On addition of the latter every urine is colored red, for this red color is caused by creatinine, which is always present in normal urine, as well as by acetone. To determine the presence of acetone aside from the creatinine add immediately a

* It must always be freshly prepared, because it does not keep very long.

very copious amount of glacial acetic acid. If the color of the liquid grows a darker red, a so-called Bordeaux red, acetone is present; if the red coloration again disappears when glacial acetic acid is added, the color was due to creatinine only.

This method for the detection of acetone does not indicate those traces of acetone which are present in every normal urine.

On standing some time the solution turns green, the creatinine, in the presence of glacial acetic acid, begins to decompose the sodium-nitroprusside. This has nothing to do with the acetone test.

It may be remarked that urines rich in acetone have a fruit-like odor, which is due to the acetone. The breath of such patients also smells of acetone. Acetone was found in urine by Petters in 1857.

Artificial Acetone Urine.—This is prepared by adding a little acetone to a healthy urine.

4. *Detection of Acetoacetic Acid.*

Dilute ferric chloride solution is added drop by drop to urine. The first drops cause a whitish precipitate of iron phosphate in any urine, additional drops a dark red (wine-red) coloration, which can be particularly seen in transmitted light.

When a urine contains only small quantities of acetoacetic acid it will sometimes appear doubtful if any

acetoacetic acid is present, since the color is then not very marked. In such cases, if the same test is made with a normal urine and the two test-tubes are compared with one another, it is not difficult to decide on the presence or absence of acetoacetic acid. Gerhard declared in 1865 that the red color of some urines on addition of iron chloride was caused by acetoacetic ester, later it was shown that it is free acetoacetic acid which imparts this color.

Since commercial acetoacetic ethyl ester gives the same test as acetoacetic acid an *artificial urine* giving this reaction can be obtained by adding a few drops of acetoacetic ethyl ester to a normal urine. They sink to the bottom, but are readily dissolved by stirring.

5. *Detection of Bile-pigment.*

Urines containing bile-pigments have a dark appearance and give a yellow foam when shaken.

For a stock solution prepare a mixture of 95 parts of a 25 per cent. solution of nitric acid with 5 parts of fuming nitric acid, and add 30 parts of water. This mixture contains a copious amount of nitrous acid for the test.

Two cubic centimeters of this mixture are placed in a test-tube and the urine is poured down the side of the inclined tube, when it will run above the acid mixture

and form a clear layer on top of this. On the border of the two liquids color zones appear, but only a green ring is decisive for bile-pigments.

This not exceedingly delicate test can be made more so in the following manner: Filter through a filter-paper larger quantities of urine in which bile-pigments are supposed to be present, or filter the same quantity of urine several times through the same filter-paper. The fibers of the paper retain the bile-pigments and repeated filtration of the same portion increases the quantity retained. After all the urine has run through the filter the latter is laid on some dry filter-paper, which will absorb the greater part of the liquid still remaining. The paper is now treated with a drop of the nitric-nitrous acid mixture on the end of a glass rod. Colored rings are formed around these spots, of which the green ring is typical of bile-pigment.

Artificial Bile-pigment Urine cannot be prepared very well. Urines to which bile-pigments, prepared from gall-stones, have been added behave differently from natural bile-pigment urine. The addition of gall is useless.

6. *Detection of Urobilin.*

Urobilin, which was discovered by Jaffé in 1868, occurs in pathological urines with bile-pigments, but also in their absence, in quantities determinable by direct

methods. Its detection has sometimes a diagnostical value.

Fill a test-tube three-fourths full with urine and acidulate with one drop of hydrochloric acid. Now add 4–5 cc. amyl alcohol. Shake the mixture carefully 6 to 8 times (otherwise an emulsion is formed). The amyl alcohol which takes up the urobilin soon separates again in a layer above the urine. Particles suspended in the alcohol are crushed with a glass rod, if necessary, and they will sink to the bottom. It will thus be possible after a few minutes to pour off about 3 cc. of the amyl alcohol into another test-tube. Dilute the alcoholic extract with double the quantity of 96 per cent. alcohol. Now add to this solution about 1 cc. of a 5 per cent. alcoholic zinc chloride solution, and then a drop of ammonia. The later neutralizes the small amount of hydrochloric acid taken up by the amyl alcoholic solution in making the extraction. The addition of this ammonia is very necessary. However, some zinc hydrate is precipitated. If this is filtered off, the solution shows a green fluorescence when urobilin was present in the urine. This fluorescence disappears when a trace of acid is added.

Artificial Urobilin Urine.—This is prepared from fresh human fæces, which always contain a considerable amount of urobilin. Pour on these 96 per cent. alcohol, avoiding an excess, in order to obtain a concentrated

solution, and filter after stirring thoroughly. The red-brown filtrate, which will keep only several months, is made slightly turbid by adding zinc chloride solution. When this turbidity is removed by a second filtration the liquid shows the green fluorescence excellently. In order to become acquainted with the reaction in urine an ample amount of the alcoholic fæces extract is added to normal urine. The latter becomes turbid from the alcohol, but this is of no consequence. Make the test as described. The green fluorescence is seen exactly as in natural urobilin urines.

If it is desired for instructive purposes to remove the turbidity which is caused by the alcoholic extract the best method is to treat the urine with some infusorial earth, to stir thoroughly and filter. The filtrate remains turbid, unless infusorial earth is used. It has as yet been impossible to remove the fæces odor which is imparted to the urine by the extract.

7. *Detection of Blood Pigment.*

Make the urine strongly alkaline by adding sodium hydrate and heat it to boiling in a test-tube. The earthy phosphates precipitated by the alkali soon subside on standing and appear colored red (by hematin) if blood was present in the urine, which cannot always be seen in the urine itself.

It may be remarked that the slight traces of blood

present and which this test does not indicate can be seen in the urinary sediment under the microscope.

Artificial Blood Urine.—This is obtained by adding some blood to a normal urine. If blood is dried by spreading it on plates and exposing it to the air it can be kept for any length of time. This dried blood can then be ground in water when needed and the filtrate poured into some normal urine.

8. *Detection of Indican.*

Pure chemistry designates as indican the plant glucoside, which yields indigo when broken up. In medicinal chemistry this name indicates the substance which by suitable oxidation of urine yields indigo. The two bodies are chemically very different. The substance occurring in urine, and which by proper treatment yields indigo, is potassium indoxyl sulphate (see below).

To test for indican, fill a test-tube half full with urine, add about 2 cc. of chloroform and fill the tube almost full with concentrated hydrochloric acid (of 25 per cent. HCl). Now add, according to Jaffé, dilute chlorated lime solution which has been recently prepared and filtered, drop by drop, shaking after each addition. The indigo formed will be dissolved by the chloroform and colors it blue. If more indigo is pro-

duced than the chloroform can dissolve it will swim as such in the liquid.

As an excess of chlorated lime again destroys the indigo which is formed at first, the following method will be more practical for those who are less experienced. It necessitates a special reagent, but one that will keep indefinitely. In this case also a test-tube is filled half full with urine and almost the same quantity hydrochloric acid, containing ferric chloride as an oxidant, is added and then likewise about 2 cc. chloroform. After shaking frequently, but not too violently (otherwise an emulsin is formed), whereby a slight warming of the solution is noticeable, the chloroform will be colored more or less blue by the indigo which is formed in the oxidation of the potassium indoxyl sulphate in proportion to the indican originally present.

The hydrochloric acid-ferric chloride solution for this test is made by dissolving 2 g. of solid ferric chloride in a half liter of hydrochloric acid, specific gravity 1.19, which is equivalent to 36 per cent. HCl. The so-called concentrated hydrochloric acid of the apothecaries which contains only 25 per cent. HCl is by far too dilute for this purpose. This reaction cannot be obtained with it.

It is perhaps not superfluous, particularly for chemists, to remark the following in regard to the cause of the occurrence of indican in urine. Albumen breaks down

in the intestinal fermentation and yields indol among many other substances. This indol is reabsorbed by the body and oxidized in the metabolic process like all similar substances. The organism combines the indoxyl which is formed from it by this oxidation immediately with sulphuric acid. This latter compound unites further with the alkali of the blood and is finally eliminated with the urine as potassium indoxyl sulphate. This is the origin of this compound, which is found in urine. It occurs in traces in every urine, of which fact any one can easily convince himself. Oxidants like chlorated lime or ferric chloride-hydrochloric acid solution * convert it into indigo.

The longer the contents of the intestines stagnate, especially those of the small intestine, the larger will be the amount of indican in the urine. Hence the diagnostical interest in its increase, which was first proven and explained by Jaffé.

Artificial Indican Urine.—It is prepared with the aid

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* The ferric chloride acts as an oxidant in such cases, a decomposition of the water taking place. Ferrous chloride is formed and hydrochloric acid and oxygen become available.

$2FeCl_3$	+	H_2O	=	$2FeCl_2$	+	$2HCl$	+	O.
Ferric chloride.		Water.		Ferrous chloride.		Hydrochloric acid.		Oxygen available for oxidizing purposes.

This is not, however, a method for generating gaseous oxygen. The equation expresses the reaction only when some substance is present in the acid ferric chloride solution which the oxygen can immediately oxidize.

of animal urine. The urine of herbivora is considerably richer in indican than human urine. The herbivora have a much longer intestine than the carnivora in order to better make use of their food, which is rich in carbohydrates and relatively poor in albumen. A much greater fermentation occurs, therefore, in the intestines of herbivora, which leads to a greater elimination of indican by the urine. If horses' urine is evaporated to dryness on the water-bath and the residue extracted with alcohol much indican passes into the alcohol. If such an extract is filtered after standing twenty-four hours, which is then easily accomplished, a solution rich in indican is obtained which will keep indefinitely, as the author has been able to verify. Only a little of this solution need be added to human urine, to become familiar with the behavior of indican urine.

9. *Sulphuric Acid.*

Sulphuric acid is detected by acidifying the urine with hydrochloric acid and adding barium chloride. Barium sulphate is precipitated, which is insoluble in all solvents.

10. *Ester Sulphuric Acids.*

To test for ester sulphuric acids in urine, add, in a test-tube, a liberal amount of barium chloride to urine.

All the sulphuric acid, besides some other substances, are precipitated. Now add one or two drops of an approximately 10 per cent. soda solution, but only enough to obtain a clear filtrate. The soda precipitates a little coarsely crystalline barium carbonate, which envelopes the fine precipitate of barium sulphate and thus prevents it from passing through the filter, which would otherwise be the case if barium chloride alone were added.

The solution thus obtained on filtration is free from sulphuric acid, but still contains the salts of the ester sulphuric acids, for the barium salts of the latter are soluble in water, as distinguished from barium sulphate.

Make the solution in the test-tube strongly acid with concentrated hydrochloric acid and boil for some time. The contents of the test-tube are hereby soon colored red by the action of the hydrochloric acid on the coloring matters of the urine and then gradually become turbid by the renewed precipitation of barium sulphate. By boiling with hydrochloric acid the ester sulphuric acids are decomposed into their components. For example, phenyl sulphuric acid (see below) is broken up, yielding phenol and free sulphuric acid, which immediately reacts with the excess of barium chloride present in the solution and is precipitated as barium sulphate. This splitting up of the ester sulphuric acids, which is the cause for the formation of free sulphuric acid, is the

reason why the renewed precipitation of barium sulphate takes place when this acid solution is boiled. It at the same time proves the presence of ester-sulphuric acids in the urine.

The explanation is as follows: Ester sulphuric acids or ethereal sulphuric acids are formed by the union of a molecule of an alcohol (in the broadest sense) with a molecule of sulphuric acid, with the elimination of a molecule of water.

$$\underset{\substack{\text{Ethyl}\\\text{(ordinary)}\\\text{alcohol.}}}{C_2H_5OH} + \underset{\substack{\text{Sulphuric}\\\text{acid.}}}{HO\cdot SO_3{*}H} = \underset{\text{Water.}}{H_2O} + \underset{\substack{\text{Ethyl sulphuric}\\\text{acid.}}}{C_2H_5O\cdot SO_3H.}$$

$$\underset{\substack{\text{Phenyl}\\\text{alcohol}\\\text{(carbolic acid).}}}{C_6H_5\cdot OH} + \underset{\substack{\text{Sulphuric}\\\text{acid.}}}{HO\cdot SO_3H} = H_2O + \underset{\substack{\text{Phenyl sulphuric}\\\text{acid.}}}{C_6H_5O\cdot SO_3H.}$$

Urines which contained phenyl sulphuric acid, of course in the form of a salt (combined with potassium or sodium), could formerly be easily obtained in chirurgical clinics when carbolic acid was much used in operations. At the present time they are rare. To obtain them for practice and instruction purposes it may be necessary to feed a large-sized dog about two grams of carbolic acid, of course in a very great dilution. He will be able to stand this dose very well and without any harm. Another method for obtaining such urines which has also been recommended is to rub in some concentrated carbolic acid on the skin of a dog.

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* Written thus for the sake of perspicuity instead of H_2SO_4.

Indican, with which we have already become acquainted in the preceding pages, is also such an ester of sulphuric acid, indoxyl sulphuric acid.

The total amount of ester sulphuric acids in normal urine is very small, so that they can hardly be detected in the quantities that can be examined in a test-tube. To find out if they are almost entirely absent is of the greatest importance in some cases. In cancer of the intestines it has been said that the operation should not be performed until the intestines are evacuated as completely as possible. This complete absence of stagnant fæces is recognized by the almost complete disappearance of ester sulphuric acids in the urine. These ester acids can hardly be present in urine after the intestines have been completely emptied, for lack of fermentation phenomena. (See the Quantitative Determination of Ester Sulphuric Acids, p. 40.)

Artificial Ester Sulphuric Acid Urine.—When no dog and a suitable cage can be had for the above purpose, such a urine can be prepared by adding a solution of potassium ethyl sulphate to a normal urine. This salt is a commercial article. It may be remarked that the decomposition of this ester acid, ethyl sulphuric acid, does not occur so rapidly when it is boiled with hydrochloric acid as that of phenyl sulphuric acid and other similar ester sulphuric acids found in urine. But potassium phenyl sulphate is not on the market and its

preparation too difficult to be recommended here, because it is very difficult to obtain good potassium pyrosulphate, which is needed for its preparation.

The ester sulphuric acids are split up into their components by boiling with hydrochloric acid, water being taken up under these conditions:

$$\underset{\text{Phenyl sulphuric acid.}}{C_6H_5O\cdot SO_3H} + \underset{\text{Water.}}{H_2O} = \underset{\text{Phenol.}}{C_6H_5OH} + \underset{\text{Sulphuric acid.}}{HO\cdot SO_3H}$$

11. *Detection of Chlorine.*

To test for chlorine acidify the urine with nitric acid and add an approximately 3 per cent. solution of silver nitrate. Silver chloride is precipitated, which can be identified by its solubility in ammonia.

The urine which has thus been made alkaline by this addition of ammonia remains, of course, turbid, silver chloride being dissolved, but the earthy phosphates precipitated. Formerly the estimation of the amount of chlorides in urine for diagnostical purposes was considered of more importance than at present.

12. *The Phosphates of Urine.*

When phosphorus is burnt it gives, as is well known, a white smoke of phosphoric anhydride. This unites with three molecules of water to form phosphoric acid, which is called ortho- or ordinary phosphoric acid:

$$P_2O_5 + 3H_2O = P_2O_8H_6, \quad \text{or halved,} \quad 2H_3PO_4.$$

The formula of ordinary phosphoric acid is therefore written H_3PO_4, since half of the formula suffices. Its salts occur in urine as sodium or potassium phosphates, in part as calcium or magnesium phosphates. These latter compounds are called earthy phosphates. Sodium (potassium) phosphate is soluble in water under all conditions. It is, therefore, not seen when urine is boiled in the albumen test or when sodium hydrate is added in making Trommer's test. The earthy phosphates behave differently. We will explain their behavior by means of the calcium salt, with which that of the magnesium salt is identical.

Phosphoric acid is tribasic, since it contains three hydrogen atoms replaceable by metals. Calcium is a bivalent metal, and by introducing one atom of calcium into two molecules of phosphoric acid the compound $Ca(H_2PO_4)_2$ is formed. We see this is an acid calcium phosphate; acid hydrogen atoms (*i.e.* replaceable by metals) are still present in the molecule. Such acid earthy phosphates are soluble in water, and it is principally this calcium phosphate which is present in urine.

When Trommer's test is to be made and sodium hydroxide is added to the urine, this alkali reacts with the acid calcium phosphate. There are formed finally neutral calcium phosphate and neutral sodium phosphate and water, according to the equations

$$\underset{\text{1 molecule acid calcium phosphate (soluble in water).}}{Ca(H_2PO_4)_2} + \underset{\text{4 mol. sodium hydrate.}}{4NaOH} = \underset{\text{1 mol. calcium sodium phosphate (insoluble in water).}}{Ca(Na_2PO_4)_2} + \underset{\text{4 molecules of water.}}{4H_2O}$$

and

$$\underset{\text{3 mol calcium sodium phosphate (insoluble in water).}}{3Ca(Na_2PO_4)_2} = \underset{\text{Breaks up into}}{} \underset{\text{1 mol. neutral calcium phosphate (insoluble in water).}}{Ca_3(PO_4)_2} + \underset{\text{4 mol. sodium phosphate (soluble in water).}}{4Na_3PO_4}$$

In neutral calcium phosphate there is no longer present a hydrogen atom which is replaceable by a metal, and neutral earthy phosphates are insoluble in water. They are precipitated from solutions as soon as the conditions exist for their formation. They are thus always precipitated from a urine when it is made alkaline, as required by Trommer's test.

The reason why the earthy phosphates in a urine are precipitated by boiling is as follows, according to Stokvis. In slightly acid urines acid calcium phosphate of the formula $CaHPO_4$ can also occur, and solutions of this water soluble calcium phosphate $CaHPO_4$ are changed by boiling into neutral calcium phosphate which is precipitated, and water soluble di-acid calcium phosphate:

$$\underset{\text{4 molecules mon-acid calcium phosphate (soluble).}}{4HCaPO_4} = \underset{\text{1 mol. diacid calcium phosphate (soluble).}}{Ca(H_2PO_4)_2} + \underset{\text{1 mol. neutral calcium phosphate (insoluble).}}{Ca_3(PO_4)_2}$$

This insoluble calcium phosphate which is precipitated is again dissolved when acetic acid is added.

13. *The Ammonia of Urine.*

Even freshly voided urine contains ammonia, which can be shown in the following manner. Pour about 25 cc. of urine into a small beaker, add milk of lime, stir and cover the beaker with a watch-glass having a piece of moistened red litmus-paper stuck on its bottom side. The paper will be colored blue after a short time by the gaseous ammonia vapors which are liberated by the milk of lime.

Sodium hydrate must not be used instead of this latter reagent, as the former decomposes the urea which is present in quantity in every urine. This decomposition occurs even in cold solution and ammonia is liberated. Lime milk does not do this. In fact urea decomposes very readily, for instance, in the decay of urine. Water is taken up and carbon dioxide and ammonia are generated:

$$\begin{matrix} & \diagup NH_2 \\ CO & \\ & \diagdown NH_2 \end{matrix} + \underset{\text{Water.}}{H_2O} = \underset{\text{Carbonic acid.}}{CO_2} + \underset{\text{Ammonia.}}{2NH_3}.$$

Urea.

These unite in turn, taking up a molecule of water, to form ammonium carbonate, on which in part the strong odor of putrefying urine depends.

According to the author's observations, the urine voided first in the morning is generally quite rich in

ammonia. This is the reason why morning urine always dissolves some copper hydroxide in Trommer's test, and on this account the ammonia is mentioned here. In this case ammonia which, as is known, also dissolves copper hydrate with a blue color is the dissolving principle. Naturally such a urine gives no clouds of yellow cuprous hydrate when heated, nor does it give any appreciable amount after it has been boiled, unless sugar is present. It cannot have a stronger reducing action than the traces of reducing substances can give which occur normally in urine. Such urines will have more of a green than blue color when only a very little ammonia is the cause of the solution of the cupric hydrate. The green color is then due to the slightly blue color uniting with the yellow of the urine to green. What has been said about urine voided in the morning in regard to its dissolving property of cupric hydrate is true to a much greater degree with decomposed urines, as they are very rich in ammonia compounds.

B.

QUANTITATIVE METHODS.

Of quantitative determinations only those of albumen, sugar, and ester sulphuric acids are used for diagnostical purposes. Approximate estimations suffice in the case of indican, etc., *i.e.* little, much, copious.

1. *Quantitative Determination of Albumen.*

The quantitative determination of albumen can of course be made most accurately by weighing the albumen precipitate, using all possible precautions. This method is employed almost exclusively for scientific purposes, but hardly for practical purposes because albumen precipitates filter badly, and the complete drying at 100° and the determination of the ash require a great deal of time, besides an analytical balance is necessary.

In practice Essbach's albuminometer is used, which is extremely convenient, but not sufficiently accurate for scientific purposes. In this instrument the quantity of albumen is read off in one-tenth per cents. on a special scale by means of the height which an albumen precipitate reaches when produced in a certain manner and allowed to stand twenty-four hours. The precipitant, Essbach's reagent, is a solution of 10 g. picric acid and 20 g. citric acid in 1 liter of water. The albuminometer—it generally costs with directions 50 cents—is nothing other than a test-tube made of strong glass having a scale and letters. The tube is filled with urine to the point U, so that the point and the meniscus of the liquid coincide, and then to the point R with the reagent. The tube is now closed with a good stopper, shaken not too violently ten or twelve times, and then

allowed to stand upright. After twenty-four hours the volume of the precipitate is read off on the special scale. The reading gives the quantity of albumen in the urine directly in tenth per cents. If urines are very rich in albumen, and the scale of the apparatus does not extend far enough for direct readings, they are diluted in the tube one-half or one-third before making the test. For this also there are graduations. The actual albumen percentage is then found by simple multiplication. If the urine which is to be tested does not react acid towards litmus-paper, it is acidified with a trace of acetic acid.

2. *Quantitative Determination of Sugar.*

1. Sugar is determined quantitatively in a purely chemical way by titration with Fehling's solution. This method is really only suitable for chemical laboratories. In the first place Fehling's solution must always be freshly prepared from its constituents, which are kept separately in the necessary concentration, since the mixture spoils on standing; secondly, and this is much more annoying, it is extremely difficult to determine the end of the reaction, for solutions of the proper strength can be bought nowadays. Only those who are engaged regularly in the titration of sugar in urine by this method can do this with complete certainty.

2. The quantitative estimation of sugar in urine by

fermentation. If a urine containing sugar is shaken with yeast the latter readily causes fermentation to set in and the sugar to break up into alcohol and carbon dioxide:

$$\underset{\text{1 molecule grape-sugar.}}{C_6H_{12}O_6} = \underset{\text{2 mol. alcohol.}}{2C_2H_6O} + \underset{\text{2 mol. carbonic acid.}}{2CO_2}$$

If the quantity of carbonic acid gas evolved in twenty-four hours is then read off in the so-called fermentation saccharometers, which can be bought cheaply everywhere, its volume is supposed to correspond to a definite amount of sugar. The saccharometers are graduated in per cents. and accompanied with full directions for use. The quantitative results of this method are very inaccurate.

3. The estimation of sugar in urine is made by far most accurately and conveniently with a polariscope in which the dextro-rotation caused by grape-sugar is read off. It is only to be regretted that such instruments are so expensive. Since albumen also effects polarized light (lævo-rotation), urines containing albumen must be freed from it in the usual way before polarization. The directions for using polariscopes—there are a number of constructions in use—need not be given here. Those instruments are most convenient which have a direct reading-scale in per cents. of grape-sugar, so that it is unnecessary to consult a table for the amount of sugar corresponding to the angle of polarization.

It is of interest to us, however, to know how to decolorize urines, for most of them are too dark to permit an accurate adjustment of the polariscope, which is necessary for taking a reading.

The author decolorizes urines as follows: ½ cc. of the very best washed blood-charcoal, as obtained commercially, is placed in a test-tube and this then almost filled with the urine and the whole shaken thoroughly. On filtering, a liquid is obtained which is generally as colorless as distilled water. The objection which was formerly urged against animal charcoal, *i.e.*, that it retained appreciable quantities of grape-sugar, and that hence by this method of decolorization the percentage of sugar in urine was reduced, can be dismissed as irrelevant when so little of such charcoal is needed.

Urines can also be clarified by shaking them with a small piece of lead acetate or by adding a solution of lead acetate or vinegar of lead. When using the last two methods the urine is diluted and this dilution must be taken into account, which is not necessary when employing animal charcoal or solid lead acetate.

3. *Determination of Total Sulphuric Acid and Ester Sulphuric Acids.*

The quantitative determination of the total sulphuric acid and ester sulphuric acids also requires several laboratory equipments and an analytical balance.

We append the method here on account of the diagnostical importance which it has obtained, and because the chemists or apothecaries, to whom the physician must in most cases refer the analysis, will hardly find the method of its determination mentioned in any one of their analytical works.

It is of interest to the physician to know not only the amount of ethereal sulphuric acids in a urine, but also its relation to the total sulphuric acid. The estimation of both varieties is made.

Total sulphuric acid is determined as follows: 50 cc. of the filtered urine are acidified strongly with concentrated hydrochloric acid (3 to 5 cc.) and heated to boiling. The liquid becomes of a dark-red color. Barium chloride solution which has been heated to boiling in a test-tube is now added; this, curiously enough, causes considerable frothing of the liquid. Hence a medium-sized beaker should be used.

The mixture is then suspended from six to eight hours in a boiling water-bath and allowed to stand over night, if possible, and filtered. Only by this procedure can the danger be avoided of the barium sulphate going through the filter, especially when the filter is first washed. The use of boiling barium chloride solution considerably minimizes this. The precipitate of barium sulphate is then treated in the usual manner.

The so-called alkaline barium chloride solution is

necessary for the determination of ethereal sulphuric acids. It is made by mixing together two volumes of a cold saturated barium hydrate solution with one volume of a cold saturated barium chloride solution. 100 cc. of this mixture is added to 100 cc. of urine. The copious precipitate that is formed soon subsides and the clear supernatant liquid is poured through a fluted filter. The filtrate now contains, of substances that concern us, only the ester sulphuric acids and, besides, considerable barium hydrate and chloride, as all the free sulphuric acid is precipitated. 100 cc. of the filtrate, which are obtained very quickly, and which represent 50 cc. of urine, are acidified strongly with hydrochloric acid and boiled for some time. The liquid acquires a dark-red color and gradually becomes turbid by the precipitation of barium sulphate whose quantity represents the amount of ester sulphuric acids. The heating is continued for six to eight hours on the water-bath and the precipitate is filtered off, preferably after allowing the solution to stand twenty-four hours. The precipitate is then washed, dried, ignited, and weighed. The ratio of ester sulphuric acids to total sulphuric acid in normal urine is about as 1 : 10.

C.

NORMAL URINE.

Normal urine is a pale-yellow or amber-colored fluid. When drink is partaken of sparingly the color can change to a red-brown on account of the greater concentration. Whether this last-mentioned color is normal or not is decided by the methods already given.

Generally fresh urine reacts acid towards litmus. This acid reaction depends upon acid salts (particularly diacid phosphate), never upon free acids.

Even fresh urine which reacts acid contains small quantities of mucous substances which on standing settle to the bottom in small clouds, and substances are always present in it which reduce an alkaline copper solution.

Urine is decomposed by the action of bacteria when standing exposed to the air, and in cases of sickness already in the bladder. It becomes alkaline, due to the decomposition of urea into carbonic acid and ammonia.

The turbidity of urines, aside from the above-mentioned unimportant slight clouds, can only be investigated microscopically. There are urines which appear not only turbid but thick (like soup). This turbidity, which often alarms persons, is caused by a copious quantity of acid sodium urate. This acid sodium urate is completely soluble at the body temperature in

the voided urine. The urine is hence voided quite clear, but on cooling the urate is precipitated. This in itself is regarded as harmless and can easily be recognized by the fact that urine which is thus turbid becomes clear again when heated in a test-tube to the temperature of the body, or when sodium hydrate is added. The acid sodium urate which is difficultly soluble in water is hereby converted into an easily soluble neutral sodium urate. The slight turbidity which is noticed after this addition of sodium hydrate is caused by earthy phosphates, which every alkaline urine precipitates (see p. 39). Pathological components can be present besides the urates in the sediment, which must be examined under the microscope in the usual manner.

Urines with strong, white, almost crystalline-looking precipitates occur less frequently. These are caused by neutral earthy phosphates. They can easily be recognized by adding several drops of acetic acid, when the turbidity disappears.

The concentration of normal urine varies between 1.002–1.030. When sugar is present in solution the specific gravity is increased to 1.040 and more. The specific gravity is determined with an areometer. These are made of a suitable length for this purpose and are called urinometers.*

* Float the instrument in some rain or distilled water at the proper temperature (generally 60° F.) and see if the zero tallies.

Normal substances occurring in urine are water, urea, uric acid, creatinine, xanthine substances, oxalic acid, ester sulphuric acids, hippuric acid, urobilin, and other coloring matters, pepsin, hydrochloric acid, sulphuric acid, phosphoric acid, sodium, potassium, ammonia, magnesium, calcium, iron. Besides the above there are a number of organic substances whose quantity only amounts to hundredths of a per cent.

II.

ANALYSIS OF STOMACH CONTENTS.

The analysis of the stomach contents which is to be given here deals with vomitings or the contents of the stomach of the patient as taken out with a stomach-pump, leaving completely out of consideration cases of poisoning. The object of the following investigation is to obtain, in cases of stomach troubles, certain reliable data for medical diagnosis by means of analysis. The chemical analysis of the stomach contents for diagnostical purposes can include only a very few substances.

The contents are always first tested for free hydrochloric acid.

1. *Test for Free Hydrochloric Acid.*

Filter the stomach contents and place a drop of the filtrate in a small porcelain dish. Now add two drops of Günzburg's reagent and warm (not heat) the porcelain dish by drawing it to and fro over a small flame, at the same time blowing over it with the mouth. As

soon as the liquid in the dish begins to dry up a beautiful red color is seen around the edge if hydrochloric acid is present, which spreads still further as the liquid dries.

Günzburg's reagent is a solution of 1 part vanilline and 2 parts phloroglucine in 30 parts of 96 per cent. alcohol. It is best kept on hand in small quantities in a small dropping-glass, which in turn is kept protected from the light in a pasteboard case. When exposed to the light the mixture gradually becomes dark-colored, but if kept in the dark it only turns wine-red, which does no harm. Besides, the alcohol evaporates much slower from the opening in the dropping-glass when it is kept in a case, and the reagent remains fit for use a year or more.

The usual methods for the detection of hydrochloric acid by testing for the chlorine in the acid cannot be used here because combined hydrochloric acid is also present in the form of chlorides, *i.e.*, sodium chloride.

Aside from the fact that Günzburg's method is the safest for determining free hydrochloric acid in the stomach contents, it affords the particular advantage over all other methods that only one drop of the filtrate is needed. This is the more important because many stomach contents filter badly.

In order to become acquainted with the behavior of a dilute hydrochloric acid solution towards Günzburg's reagent a .2 per cent. solution is used (about as

strong as the hydrochloric acid in a normal stomach). This solution may be further diluted for practice until the limit of sensibility of the reaction is reached.

2. *Detection of Lactic Acid.*

Add to the filtered gastric juice which fills a test-tube about one-fourth full, several cubic centimeters of a ferric chloride solution which has been diluted in a test-tube until the color is hardly visible. If this addition gives a canary-yellow color to the mixture lactic acid is present. Should no lactic acid be detected in this way the reason for this may be that it is present in too small quantity in the gastric juice for this direct method. In this case fill a test-tube three-fourths full with the filtered juice, add some ether and shake thoroughly; an emulsin will hardly ever be formed. When the ether, which contains the lactic acid, has separated, pour it off into a small porcelain dish, pour fresh ether into the test-tube, and repeat this procedure two more times. The ether in the dish is evaporated on a water-bath. Since the quantity of ether is small the dish may also be placed on a double-wire gauze and a very small flame placed under it. Care must be taken that it does not catch fire. The residue remaining after evaporating the ether must not be overheated (burnt). The last trace of ether is hence evaporated by blowing on it with the mouth. Two or three drops of water are

added to the residue and the diluted ferric chloride solution added. The presence of lactic acid will be proven in this case also by the appearance of the yellow color, which is due to iron lactate. This can be seen very well on the white background of the dish. The absence of lactic acid is shown by the absence of a yellow color.

A lactic acid solution containing about .2 per cent. of the acid is used for practicing the reaction, and can be diluted further.

If hydrochloric acid has been found in the stomach contents, one can be satisfied with this favorable result. If the acid is absent and lactic acid is found instead, or is also not present and only volatile acids are present (see below), a microscopic investigation in addition to the chemical analysis is indispensable (particularly for yeast, sarcinæ, bacilli).

3. *Detection of Volatile Acids.*

Filter the gastric juice in which neither hydrochloric nor lactic acid has been found and distill as much of the filtrate as can be obtained. The distillate is tested with litmus-paper for acid reaction. The first drops will give this reaction if volatile acids are present; it is therefore not necessary to continue long the distillation. A whole series of volatile acids (formic, etc.) distills over, which cannot be separated, nor is this

necessary. Butyric acid particularly shows its presence by its sweaty odor. To practice this last reaction add of course a few drops of formic acid, butyric acid, etc., to some water and distill.

Artificial Stomach Contents.—After these reactions have been learned, it is preferable to make the tests with artificial stomach contents instead of with dilute acids. To prepare these contents dissolve some commercial peptone in water (peptone is albumen which has become soluble in water by being digested). Such a solution foams considerably when shaken and does not become clear by filtering. This is a property of peptone and explains why gastric juice gives no clear filtrate. It becomes clear when alkali is added, *i.e.*, as required by Trommer's test. To the peptone solution add some grape-sugar solution and thereupon some hydrochloric acid, lactic acid, and volatile acids according to the tests that are to be made. The observation will be made that slight quantities of added hydrochloric acid cannot be detected with even so delicate a reagent as that of Günzburg's. The reason for this is that the peptone unites with the hydrochloric acid to form a compound in which the latter is not detectable as free acid. Hence the stomach contents of a patient should not be tested shortly after a meal rich in albumen, or after milk has been drunk; the albumen of the latter is very quickly peptonized in the stomach. Several hours should

intervene after such a meal. After this time a normal stomach will again contain free hydrochloric acid.

If Trommer's test is applied to the gastric juice a violet solution is first obtained, and this coloration establishes the presence of peptone, since it is caused by it.

If this alkaline liquid containing cupric oxide is boiled, red cuprous oxide, but never yellow cuprous oxide, is precipitated, since grape-sugar is present. The grape-sugar in natural gastric juice can have been eaten as such or the ptyalin of the saliva has made it from starch. The gastric juice does not invert starch; this process occurs in the intestinal digestion. The violet color caused by the presence of peptone is not changed by boiling.

4. *Test for Absence of Pepsin.*

If hydrochloric acid has been found in a stomach content a poor digestion may be due to a lack of pepsin, for normal digestion is the mutual action of these two substances. To decide whether there is a lack of pepsin, which is said to be extremely rare, proceed as follows:

Divide the filtered gastric juice into three parts and place in each a piece of fibrin. No. 1 is used for a blank determination, No. 2 receives some .2 per cent. hydrochloric acid, and No. 3 some commercial pepsin.

All three samples are placed in a warming-closet heated to 38°–40°. In the course of an hour it can be easily discerned which portion has dissolved the most fibrin, *i.e.*, digested it. If this is the case with the sample to which pepsin was added there was a lack of pepsin in the original gastric juice.

Fibrin is used because this is the most easily digestible solid albumenoid for artificial digestion experiments. It is obtained by allowing fresh blood to stand a short time. A solid red-colored mass is soon separated from it, which the blood is capable of retaining in solution only so long as it circulates in a living body. When this mass is washed with water it loses its red color and has been given the name fibrin. As fibrin soon decomposes, it is kept best by pouring on it in a glass some water containing considerable glycerine. Thus treated it will keep a long time. Before use the glycerine is washed out with water. Finely cut slices of a hard-boiled egg can be used when no fibrin can be had, but it is digested much more slowly. It does not come within the scope of this book to test for the propeptone and peptone which are formed from the albumenoids by such digestions, for such a test cannot claim any particular diagnostical value.

REAGENTS AND APPARATUS.

ALPHABETICAL LIST OF ALL THE NECESSARY REAGENTS.

Acetic acid (10 per cent.).
Acetoacetic ethyl ester.
Acetone.
Alcohol.
Ammonia.
Amyl alcohol.
Animal charcoal.
Barium chloride (10 per cent. and cold saturated solution).
Barium hydrate solution (cold saturated).
Blood.
Butyric acid.
Calcium chloride solution.
Chlorated lime solution (very dilute).
Chloroform.
Citric acid.
Copper sulphate solution (5 per cent.).
Ether.
Fæces.
Ferric chloride (solid and 5 per cent. solution).
Fibrin, or egg-albumen.
Glacial acetic acid.
Grape-sugar.
Horses' urine

Hydrochloric acid (25 per cent. and 36 per cent.).
Infusorial earth.
Lactic acid.
Lime milk.
Nitric acid (25 per cent. and fuming).
Pepsin.
Peptone.
Phloroglucine.
Picric acid.
Potassium ethyl sulphate.
Silver nitrate solution (3 per cent.).
Sodium carbonate solution (10 per cent.).
Sodium hydrate (10 per cent. solution).
Sodium nitroprusside.
Vanilline.
Zinc chloride solution (alcoholic 5 per cent.).

REAGENTS NECESSARY FOR URINARY ANALYSIS.

General preservation of urines ...	Chloroform.
Albumen test	10 per cent. acetic acid. Infusorial earth.
Artificial albumen urine	Egg-albumen.
Artificial earthy phosphate urine.	Calcium chloride solution. Soda solution.
Sugar test	10 per cent. sodium hydrate solution. 5 per cent. copper sulphate solution.
Artificial sugar urine	Grape-sugar solution. Sodium nitro-prusside.
Acetone test	10 per cent. sodium hydrate solution. Glacial acetic acid.
Artificial acetone urine.....	Acetone.
Acetoacetic acid test	5 per cent. ferric chloride solution.
Artificial acetoacetic acid urine. .	Acetoacetic ethyl ester.
Bile-pigment test	95 parts of 25 per cent. nitric acid mixed with 5 parts fuming nitric acid and 30 parts water.

Urobilin test	Hydrochloric acid.
	Amyl alcohol.
	96 per cent. alcohol.
	5 per cent. alcoholic zinc chloride solution.
	Ammonia.
Artificial urobiline urine	Fæces.
	96 per cent. alcohol.
Blood test	10 per cent. sodium hydrate solution.
Artificial blood urine	Fresh or dried blood.
Indican test	Dilute chlorated lime solution.
	25 per cent. hydrochloric acid, (or)
	Solid ferric chloride.
	36 per cent. hydrochloric acid.
	Chloroform.
Artificial indican urine	Horses' urine.
	96 per cent. alcohol.
Sulphuric acid test	10 per cent. barium chloride solution.
	Hydrochloric acid.
Ester sulphuric acids	10 per cent. barium chloride solution.
	10 per cent. soda solution.
	25 per cent. hydrochloric acid.
Artificial urine with ester sulphuric acids	Potassium ethyl sulphate.
Chlorine test	Nitric acid.
	3 per cent. silver nitrate solution.
Ammonia test	Milk of lime.

REAGENTS AND APPARATUS REQUIRED FOR QUANTITATIVE URINARY ANALYSIS, ETC.

Determination of albumen	Essbach's albuminometer.
	Essbach's reagent, 5 g. picric acid, 10 g. citric acid dissolved in one-half liter of water.

Determination of sugar.....	Fermentation saccharometer.
	Polarizing apparatus.
	Animal charcoal.
Determination of total sulphuric acid and ester sulphuric acids ..	25 per cent. hydrochloric acid.
	10 per cent. barium chloride solution.
	Alkaline barium chloride solution (mixture of two parts of cold saturated barium hydrate solution and one part cold saturated barium chloride solution).
Determination of the specific gravity.....	Ureometer.

REAGENTS NECESSARY FOR THE ANALYSIS OF THE STOMACH CONTENTS.

Test for hydrochloric acid	Günzburg's reagent.
	1 part vanilline and 2 parts phloroglucine in 30 parts of 96 per cent. alcohol.
Test for lactic acid.....	Very dilute ferric chloride solution.
	Ether.
Artificial stomach contents	Peptone.
	Grape-sugar.
	Hydrochloric acid.
	Lactic acid.
	Butyric acid.
Digestion experiment.....	Filtered gastric juice.
	0.2 per cent. hydrochloric acid.
	Pepsine.
	Fibrine (or hard-boiled egg).

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